Supplementary Information

Caught in Action: Selecting Peptide Aptamers Against Intrinsically Disordered Proteins in Live Cells

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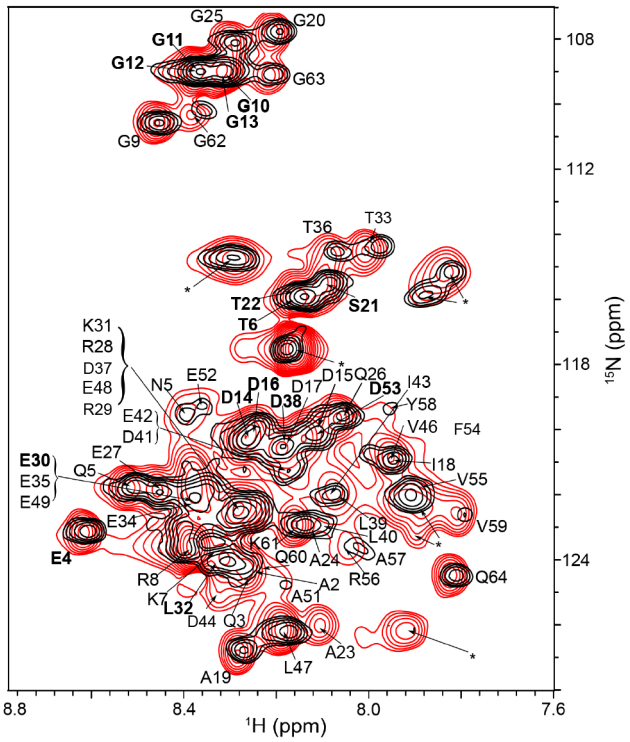
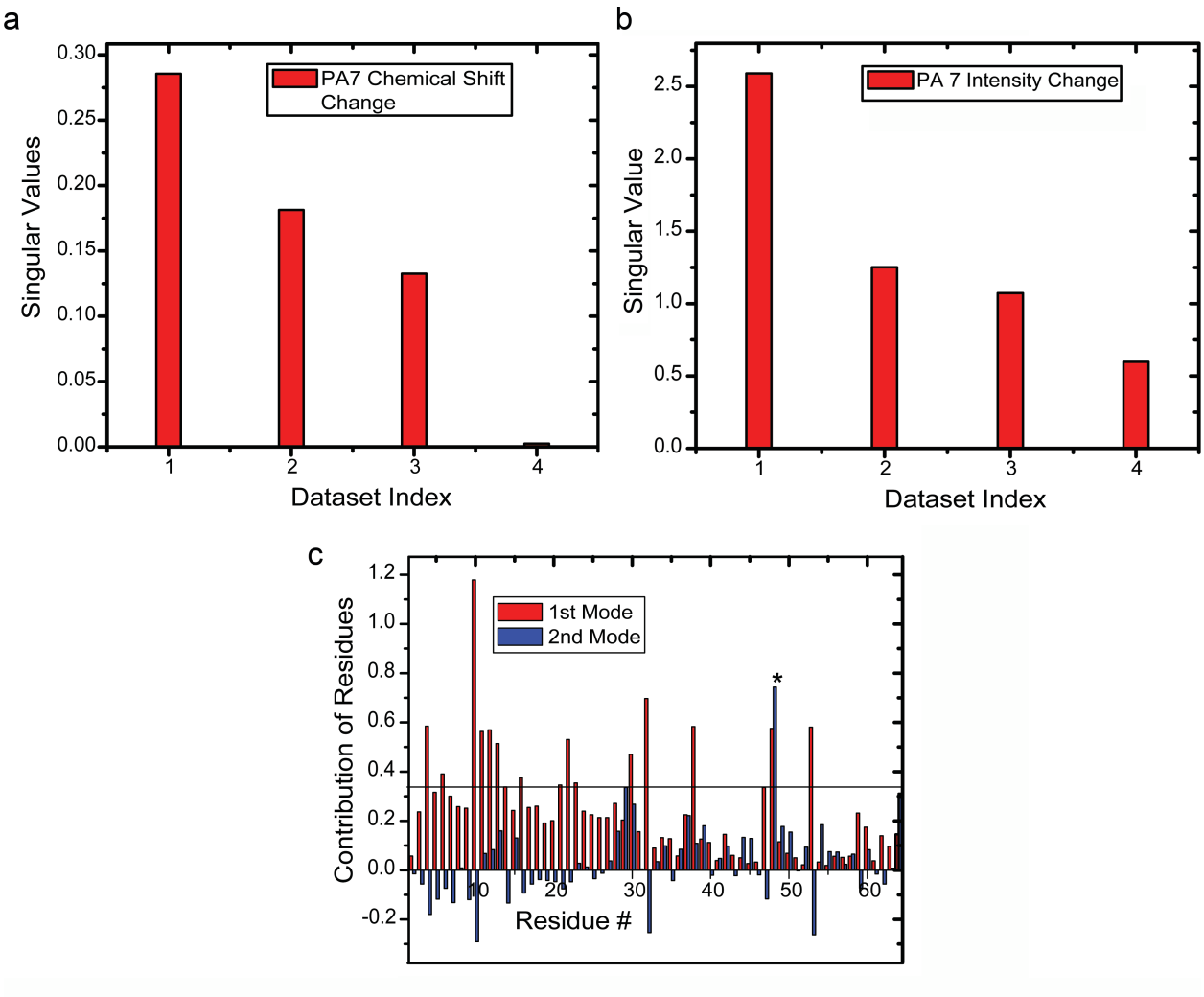
List of Figures.

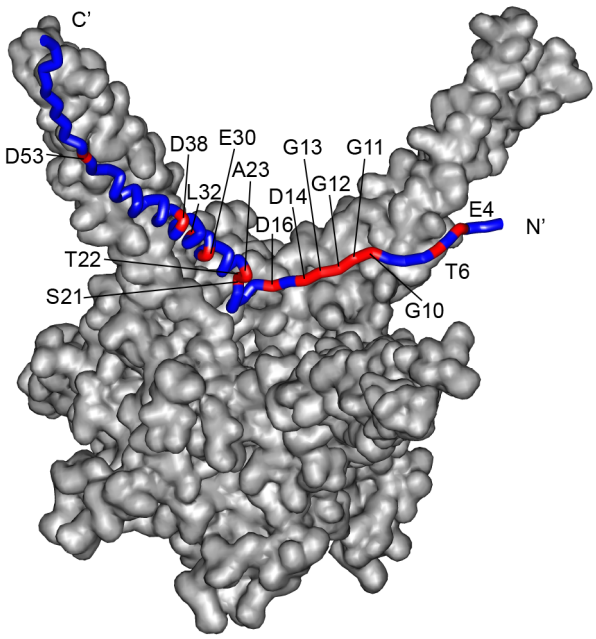


Supplementary Figure 1. In-cell NMR of [*U*-^{15}N] Pup-PA-7. (a) ^{1}H{^{15}N}-HSQC spectrum of *E. coli* after 2 h of [*U*-^{15}N] Pup over-expression (red), overlaid with spectrum (black) of *E. coli* after 2 h of [*U*-^{15}N] Pup over-expression followed by approximately 16 h over-expression of PA-7. Due to ^{15}N editing, only backbone amide protons and nitrogens of Pup are present in the spectrum. Most peaks do not change their positions indicating that only a subset of Pup residues interact with PA-7 (bold). The sharp peaks in the spectrum, which correspond to various metabolites of [*U*-^{15}N] ammonium ion, are labeled with asterisks.

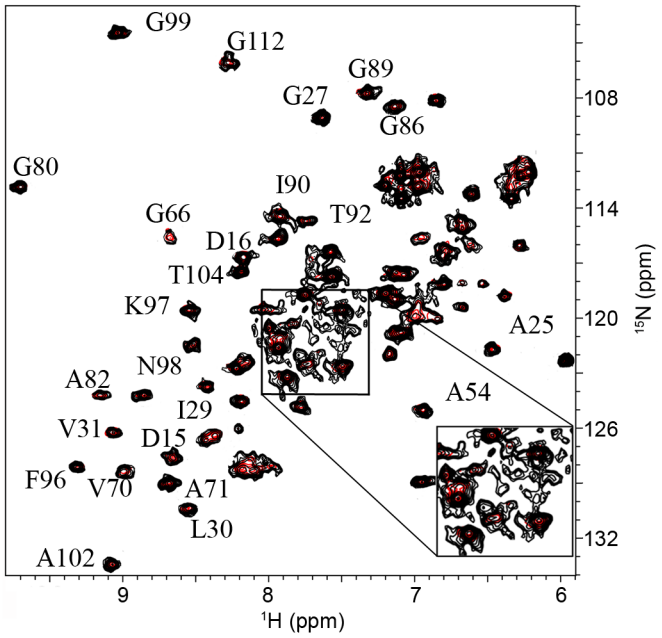


Supplementary Figure 2. SVD analysis of Pup-PA-7 binding. Matrices consisting of either chemical shift changes, MCSC, or intensities changes, MIC, in the in-cell [*U*-^{15}N] Pup peaks over the time course of PA-7 overexpression were analyzed[1] to identify Pup residues involved in PA binding. The scree plots (**a** and **b**) show the distribution of singular values that define the relative contribution of each binding mode to the MCSC or MIC, respectively. **(a)** The first binding mode of Pup-PA-7 contributes to 61.8% of MCSC, respectively, with no clear drop in singular values, precluding us from identifying principal binding modes in this case. The R^2

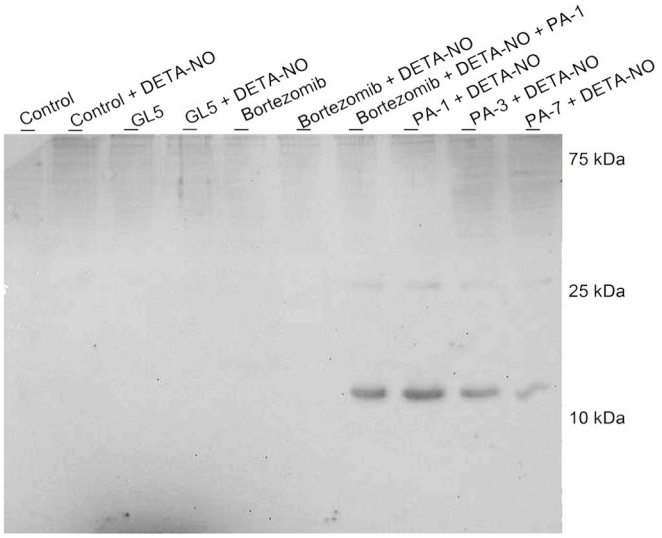
value of the scree plot linear regression is 0.97. **(b)** The first binding mode of Pup-PA-7 contributes to 68.5% of MIC, respectively, indicating that it is a potential principal binding mode. A clear drop in the progression of singular values is evident after the first singular value. The R^2 value of the scree plot linear regression is 0.79. **(c)** The contribution of Pup residues to the first and second binding modes with PA-7 is shown in red and blue bars, respectively. The maximum contribution of Pup residues to the second mode is used as a threshold to identify Pup residues affected by PA-7 binding. Proximity to a metabolite peak generates an anomalously large contribution to the second binding mode by E48 indicated by an asterisk.



Supplementary Figure 3. **Pup residues (shown in red) affected by the interaction with PA-7 are similar to that of PA-3.** Based on the SVD analysis of in-cell NMR data, the negatively charged N-terminal tail and a few residues of the α-helix that interact with Mpa are affected by the binding of PA-7. Some labeled residues are obscured due to image orientation. The image of the Mpa-Pup structure (PDB code 3M9D[2]) was constructed by using Modeller[3].



Supplementary Figure 4. Only the PA loop is involved in PA-7 binding to Pup. A $^{1}H\{^{15}N\}$-HSQC spectrum of purified [*U*-^{15}N]-PA (red), overlaid with a spectrum (black) obtained after titrating with purified Pup. The insert shows the residues from the PA-7 loop. Well-resolved peaks of the thioredoxin scaffold are labeled. Most peaks do not change their positions or intensities reflecting the fact that thioredoxin is a neutral PA scaffold. Only a subset of PA residues, from the PA loop, exhibit substantial or complete broadening of peaks, indicating Pup-PA-7 loop interaction. Due to ^{15}N editing, only backbone and side chain amide protons and nitrogens of PA are present in the spectra.



Supplementary Figure 5. Expression of PAs in BCG. Expression of PAs in BCG was verified by Western blot analysis. Lysates from *M. bovis* BCG cultures were probed for thioredoxin. Thioredoxin was not detected in the control cultures (lanes 1-6) and was detected at ~12 kDa in the cultures in which PAs were expressed (lanes 7-10).

1. Majumder, S., DeMott, C.M., Burz, D.S. & Shekhtman, A. Using singular value decomposition to characterize protein-protein interactions by in-cell NMR spectroscopy. *Chembiochem* **15**, 929-33 (2014).
2. Wang, T., Darwin, K.H. & Li, H. Binding-induced folding of prokaryotic ubiquitin-like protein on the Mycobacterium proteasomal ATPase targets substrates for degradation. *Nat Struct Mol Biol* **17**, 1352-7 (2010).
3. Eswar, N., Eramian, D., Webb, B., Shen, M.Y. & Sali, A. Protein structure modeling with MODELLER. *Methods Mol Biol* **426**, 145-59 (2008).